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EFFECTS OF AGE AND GROUP SIZE ON HABITAT SELECTION AND ACTIVITY LEVEL IN *RANA PIPIENS* TADPOLES

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Amphibian larvae may use both intrinsic cues – such as their own age, size or developmental stage – and extrinsic cues – such as chemical cues and group size – to make behavioural decisions. We performed a laboratory experiment to study habitat selection and activity level of *Rana pipiens* tadpoles at different ages and in groups of different sizes. Two ages of tadpoles were used: early (2 weeks after hatching; Gosner stage 25) and late (8 weeks after hatching; Gosner stages 36-38). Three group sizes were used: one, two and six. Tadpoles in pairs or groups tended to be more active and to occur in the open water column more than lone tadpoles. Younger tadpoles tended to use the open water column less and to be less active than older tadpoles, but there was no effect on the use of the vegetated habitat. Our results indicate that the determinants of amphibian larval behaviour include responses to both intrinsic (body size, age) and extrinsic (group size) cues.

Key words: activity, age, group size, habitat selection, *Rana pipiens*, tadpoles

INTRODUCTION

Amphibians appear to use cues (direct and indirect) to determine the appropriate behaviour for any given situation. The possibility exists that amphibian larvae use both intrinsic cues (e.g. their own age, size or developmental stage) and extrinsic cues (e.g. chemical cues and group size) to make behavioural decisions. For example, the presence of predators affects the behaviour of amphibian larvae in numerous ways (e.g. Anholt & Werner, 1995; Kupferberg, 1998; Lefcort, 1998). However, changes in age, size or developmental stage, or changes in the number of larvae in a given area, could alter the perception of predation risk, thus altering the behaviour of the larvae. Other factors potentially affecting tadpole activity and habitat selection include oxygen concentration, population density, water temperature, water depth, vegetation density, predators, time of day, and substrate type or pattern (Noland & Ultsch, 1981; Waringer-Löschenkohl, 1988; Johnson, 1991; Peterson, Bull & Wheeler, 1992; Kiesecker & Blaustein, 1998; Schley, Griffiths & Román, 1998; Nie, Crim & Ultsch, 1999). Understanding how behaviour (e.g habitat selection, activity level) is determined, and what cues or factors influence this determination, is important for understanding the link between an individual's behaviour and the consequences of that behaviour on individual performance, population dynamics or community processes.

We performed an experiment to assess the effect of age and group size on habitat selection and activity level of northern leopard frog (*Rana pipiens*) tadpoles. Since amphibian larvae often respond to predator cues in such a way as to reduce predation risk (e.g. Anholt & Werner, 1995; Kupferberg, 1998; Lefcort, 1998; including the southern leopard frog, *Rana utricularia*, Lefcort, 1996), we hypothesized that tadpoles may alter their behaviour in response to an intrinsic cue – age/body size – and an extrinsic cue – group size – in a manner consistent with adaptive anti-predator responses. The susceptibility of anuran larvae to predation often decreases with size (e.g. Semlitsch, 1990), thus we expected younger, smaller tadpoles to use habitats that may be perceived as less risky (e.g. increased use of a vegetated habitat, decreased use of the open water column) and to lower their activity level, decreasing predation risk (e.g. Skelly, 1994; Kupferberg, 1998). We predicted that tadpoles alone or in small groups would use less risky habitats and have lower activity levels when compared to tadpoles in larger groups, since the size of the group may influence the perception of predation risk – the larger the group the lower the individual's risk (e.g. Watt, Nottingham, & Young, 1997). We also expected that the effect of group size would decrease in older, larger tadpoles since the perception of risk would be lower; thus group size may not have any additional effect (i.e. we predicted a significant interaction term).

Younger and smaller tadpoles might also be predicted to swim less and use the open water column less simply because they may be less skilled or powerful swimmers than older and larger tadpoles (e.g. Chovanec, 1992; Brown & Taylor, 1995; Jung & Jagoe, 1995; McCollum & Leimberger, 1997). However, if this were the case we would expect no interaction term between group size and age class (i.e. young tadpoles are poor swimmers regardless of what size group they are in). Our controlled experimental design allows us to ignore possible explanations based on food distribution, temperature variability and oxygen availability.

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TABLE 1. MANOVA table for the effects of age/developmental stage and group size on the behavior of *Rana pipiens* tadpoles. For univariate tests, α=0.017.

(1) MULTIVARIATE TEST

	Wilks' Lambda	df	F	P
Age class	0.13	3,16	35.70	<0.0001
Group size	0.22	6,32	6.12	0.0002
Age class x group size	0.45	6,32	2.62	0.04

(2) UNIVARIATE TESTS

	df	F	P
Use of open water column			
Age class	1	72.05	<0.0001
Group size	2	9.12	0.002
Age class x group size	2	9.12	0.002
Error	18		
Use of vegetated habitat			
Age class	1	0.01	0.93
Group size	2	1.88	0.18
Age class x group size	2	0.03	0.97
Error	18		
High activity level			
Age class	1	8.75	0.01
Group size	2	8.87	0.003
Age class x group size	2	0.002	1.00
Error	18		

MATERIALS AND METHODS

Rana pipiens range across the northern part of the United States and the southern half of Canada (Conant & Collins, 1991). They do not appear to form aggregations in the field or the laboratory (Wassersug, 1973). *Rana pipiens* is also apparently palatable to fish (Kruse & Francis, 1977). Other likely predators include macroinvertebrates such as immature odonates.

Rana pipiens eggs were obtained from the Carolina Biological Supply Company. Eggs were incubated at room temperature (19°C). Eggs hatched after 10 days of incubation. After hatching, tadpoles were kept in opaque plastic tubs in aerated, filtered, non-chlorinated water in a room maintained at around 19°C and on a 12L:12D photoperiod. Tadpoles were fed and containers cleaned daily.

Test arenas (3) were 19 litre aquaria each with a shallow bed of pebbles covering the bottom, with two larger rocks (20 - 30 cm in diameter, 2 - 4 cm thick) at one end of the tank to provide potential shelter and contrast with the gravel, and artificial vegetation (a mix of standard plastic aquarium plants; three "plants" per arena) placed at the other end of the tank; leaving an open area in the centre. The test arenas were filled half-full (9 litres) with filtered, non-chlorinated water (at room temperature c. 19°C). Lighting of the test arenas was achieved by hanging fluorescent lights about 20 cm above the tops of the aquaria. These lights were longer than the aquaria thus giving equal light to the entire test arena. After each trial, test arenas were emptied, cleaned and refilled to eliminate any potential chemical cues from previous tadpole(s).

The first set of trials (early) occurred two weeks after hatching (8-12 December 1997) when all tadpoles were free swimming (stage 25; Gosner, 1960). The second set of trials (late) occurred six weeks after the completion of the first set of trials (eight weeks post-hatching; 22-29 January 1998). Tadpoles in the second set of trials were stages 36-38 (Gosner, 1960). Tadpoles differed substantially in size between trials. Within each set of trials tadpoles were matched for size, and any tadpole that differed from the others in size or developmental stage was not used. Individual tadpoles were used only once in the entire study.

Each trial was begun by carefully placing the appropriate number of tadpoles (one, two or six) in the middle of the test arena and allowing 15 min for tadpoles to acclimate to the arena. Following the acclimation period, the position and activity level for each tadpole were noted at one minute intervals for 15 min (i.e. 15 observations per trial). Specific information collected included: (1) location in the tank (bottom = resting on the substrate or against walls of the aquarium, open = floating or swimming in water column); (2) habitat (rock, neutral, vegetated); and (3) activity level (low = no movement, medium = some tail movement, high = actively swimming). Four replicates were performed for each tadpole density. Trials were typically run in the afternoon. Tadpoles were not fed during the trials; however, they did have constant access to food in their holding containers prior to a trial.

To analyse the data, we generated a mean proportion of tadpoles observed in each location, habitat, or at each activity level for a given trial, by averaging the proportions of tadpoles from each of the 15 observations per trial. For example, if all tadpoles were in the vegetation for 10 of the 15 observations and in the neutral area for the remaining five observations, the mean proportion in the vegetation would be 0.67, and the mean proportion in the neutral area would be 0.33. Thus for each trial we had a mean proportion for each location (bottom, open), habitat (rock, neutral, vegetated) and activity level (low, medium, high). Prior to analysis we transformed all proportion data with an arcsin square-root transformation.

Since the mean proportions within a behavioural category (e.g. location, habitat and activity) are not independent, we chose to use only a single variable in each category in the statistical analyses. Variables analysed were (1) use of open water column, (2) use of vegetated habitats, and (3) high activity levels (analyses with the other variables gave qualitatively similar results). These variables were chosen for analysis because they closely reflect our predictions: increased use of open water column and high activity levels would suggest increased use of risky habitats, whereas increased use of the vegetated area would suggest decreased use of risky habitat. We used a MANOVA to determine if any overall effect of the independent variables existed, and then univariate ANOVAs to test each behavioral category if the MANOVA indicated significant effects (see Scheiner, 1993).

RESULTS

Overall, there was a significant effect (α=0.05) of age class and group size (MANOVA; Table 1). The interaction between age class and group size was also significant. Since there were significant multivariate effects we conducted univariate tests on each behavioural category (corrected α = 0.05/3 = 0.0167).

Early tadpoles never used the open water column (Table 1; Fig. 1A). Lone tadpoles were less likely to be in the open water column than tadpoles in pairs or groups (Table 1; Fig. 1A). The difference between early and late tadpoles' use of the open area increased between single and multiple individuals (Table 1; Fig. 1A). The proportion of lone individuals using the open water column was similar for both early and late stage tadpoles, whereas for pairs and groups of six, late stage tadpoles used the open water column more than early stage tadpoles.

Age class and group size did not affect tadpole use of the vegetated habitat (Table 1). The interaction term was also not significant.

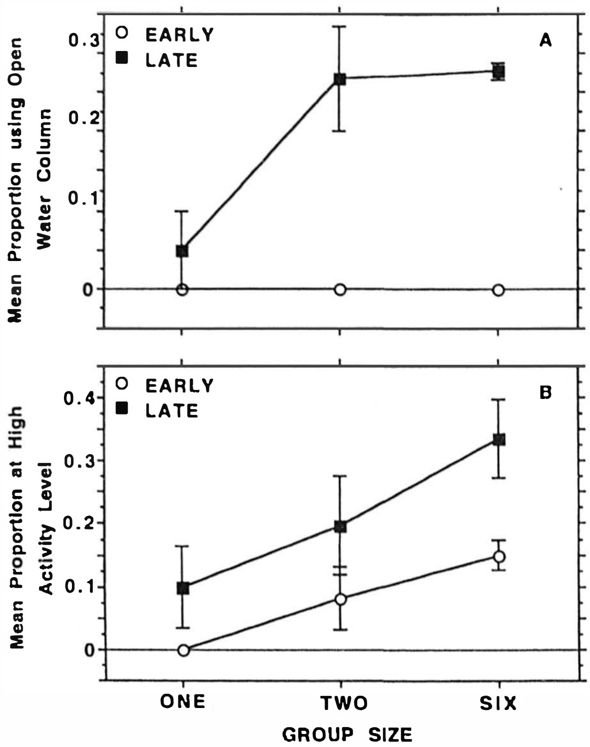

FIG. 1. The effect of age class and group size on mean (±1 SE) proportion of observations of *Rana pipiens* tadpoles (A) in the open water column of the test arena, and (B) at high activity levels (actively swimming). Values are untransformed means.

Late tadpoles showed high activity levels more often than early tadpoles (Table 1; Fig. 1B). The proportion of time tadpoles spent at high activity level increased with group size for both early and late tadpoles (Table 1; Fig. 1B). The interaction between age and group size was not significant.

DISCUSSION

In our experiment, *R. pipiens* tadpoles altered their behaviour in response to extrinsic (group size) and intrinsic (age, stage or size) cues. Tadpoles in pairs or groups were more likely to be active and in the open water column than lone tadpoles that tended to be inactive and resting on the bottom.

Early tadpoles tended to stay at the bottom of the test arena and be less active than late tadpoles. However, early and late tadpoles did not differ in their use of vegetated habitats. Our results are similar to those from other studies. Puttlitz *et al.* (1999) found that *Hyla regilla* tadpoles changed their response to predators as they grew: tadpoles increased activity in the presence of a predator as they got bigger. Warkentin (1999) also found changes in antipredator responses with age in *Agalychnis callidryas* tadpoles, including microhabitat use and activity. Other studies have shown that responses to predator cues change with age in some anuran larvae (e.g. Brown & Taylor, 1995; Bridges & Gutzke, 1997). Large tadpoles may reach a size where they are no longer able to be eaten by a gape-limited predator (see Caldwell, Thorp, & Jervey, 1980;

Semlitsch, 1990); thus larger tadpoles may use riskier, more rewarding habitats or behaviours.

Our results might also be explained by differences in swimming performance between the early and late tadpoles. The younger tadpoles may be weaker swimmers (e.g. Chovanec, 1992; Brown & Taylor, 1995; Jung & Jagoe, 1995; McCollum & Leimberger, 1997), and thus swim less and use the open water less than older tadpoles. Indeed, the early tadpoles in our experiment had just become free swimming and may have avoided or not been able to exploit as well, habitats requiring active swimming – such as the open water column. This does not, however, explain the difference in the use of the open water column of older tadpoles in pairs and groups of six, and lone tadpoles (see Fig. 1A), nor does it explain the increase in high activity with group size in both early and late tadpoles (see Fig. 1B).

Single tadpoles avoided more risky habitats and behaviours, such as the open water column and high activity levels. Our results are similar to those of other researchers investigating anuran larvae. *Phrynomantis microps* tadpoles in a natural pond use vegetated habitats more when in small groups, whereas they use open water more when in large groups (Rödel & Linsenmair, 1997). Increasing group size increases activity level in some anuran larvae, both in the presence (Lefcort, 1998) and absence (Griffiths & Foster, 1998) of predator cues. Our results appear to be consistent with what would be expected if such behavioural shifts were selected to avoid predation. Larger groups may afford some degree of protection for individuals from predation (e.g. Watt *et al.*, 1997). Thus, individuals in larger groups may be more active, which is often beneficial as it facilitates foraging and may ultimately increase growth (e.g. Skelly & Werner, 1990; Kupferberg, 1998). Another explanation is that tadpoles in larger groups perceive the possibility of increased competition and increase activity and swimming (and thus also use the open water column) to increase foraging. Higher tadpole activity levels appear to be a characteristic of superior competitors (e.g. Werner, 1992, 1994), and activity level increases in low food situations where competition might be expected (e.g. Anholt & Werner, 1995).

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REFERENCES

Anholt, B. R. & Werner, E. E. (1995). Interaction between food availability and predation mortality mediated by adaptive behavior. *Ecology* **76**, 2230-2234.

Bridges, C. M. & Gutzke, W. H. N. (1997). Effects of environmental history, sibship, and age on predator-avoidance responses of tadpoles. *Can. J. Zool.* **75**, 87-93.

Brown, R. M. & Taylor, D. H. (1995). Compensatory escape mode trade-offs between swimming performance and maneuvering behavior through larval ontogeny of the wood frog, *Rana sylvatica*. *Copeia* **1995**, 1-7.

Caldwell, J. P., Thorp, J. H. & Jervey, T. O. (1980). Predator-prey relationships among larval dragonflies, salamanders, and frogs. *Oecologia* **46**, 285-289.

Chovanec, A. (1992). The influence of tadpole swimming behaviour on predation by dragonfly nymphs. *Amphibia-Reptilia* **13**, 341-349.

Conant, R. & Collins, J. T. (1991). *A Field Guide to Reptiles and Amphibians of Eastern and Central North America, Third Edition.* Houghton Mifflin Company, Boston.

Gosner, K. L. (1960). A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica* **16**, 183-190.

Griffiths, R. A. & Foster, J. P. (1998). The effect of social interactions on tadpole activity and growth in the British anuran amphibians (*Bufo bufo*, *B. calamita*, and *Rana temporaria*). *J. Zool.* **245**, 431-437.

Johnson, L. M. (1991). Growth and development of larval northern cricket frogs (*Acris crepitans*) in relation to phytoplankton abundance. *Freshw. Biol.* **25**, 51-59.

Jung, R. E. & Jagoe, C. H. (1995). Effects of low pH and aluminum on body size, swimming performance, and susceptibility to predation of green tree frog (*Hyla cinerea*) tadpoles. *Can. J. Zool.* **73**, 2171-2183.

Kiesecker, J. M. & Blaustein, A. R. (1998). Effects of introduced bullfrogs and smallmouth bass on microhabitat use, growth, and survivorship of native red-legged frogs (*Rana aurora*). *Conserv. Biol.* **12**, 776-787.

Kruse, K. C. & Francis, M. G. (1977). A predation deterrent in larvae of the bullfrog, *Rana catesbeiana*. *Trans. Amer. Fish. Soc.* **160**, 248-252.

Kupferberg, S. J. (1998). Predator mediated patch use by tadpoles (*Hyla regilla*): risk balancing or consequence of motionlessness? *J. Herpetol.* **32**, 84-92.

Lefcort, H. (1996). Adaptive, chemically mediated flight response in tadpoles of the southern leopard frog, *Rana utricularia*. *Copeia* 1996, 455-459.

Lefcort, H. (1998). Chemically mediated fright response in southern toad (*Bufo terrestris*) tadpoles. *Copeia* **1998**, 445-450.

McCollum, S. A. & Leimberger, J. D. (1997). Predator-induced morphological changes in an amphibian: predation by dragonflies affects tadpole color, shape, and growth rate. *Oecologia* **109**, 615-621.

Nie, M., Crim, J. D. & Ultsch, G. R. (1999). Dissolved oxygen, temperature, and habitat selection by bullfrog (*Rana catesbeiana*) tadpoles. *Copeia* **1999**, 153-162.

Noland, R. & Ultsch, G. R. (1981). The roles of temperature and dissolved oxygen in microhabitat selection by the tadpole of a frog (*Rana pipiens*) and a toad (*Bufo terrestris*). *Copeia* **1981**, 645-652.

Peterson, A. G., Bull, C. M. & Wheeler, L. M. (1992). Habitat choice and predator avoidance in tadpoles. *J. Herpetol.* **26**, 142-146.

Puttlitz, M. H., Chivers, D. P., Kiesecker, J. M. & Blaustein, A. R. (1999). Threat-sensitive predator avoidance by larval Pacific treefrogs (Amphibia, Hylidae). *Ethology* **105**, 449-456.

Rödel, M.-O. & Linsenmair, K. E. (1997). Predator-induced swarms in the tadpoles of an African savanna frog, *Phrynomantis microps*. *Ethology* **103**, 902-914.

Scheiner, S. M. (1993). MANOVA: Multiple response variables and multispecies interactions, pp. 94-112. In: *Design and Analysis of Ecological Experiments*. S.M. Scheiner and J. Gurevitch (eds.). Chapman and Hall, New York.

Schley, L., Griffiths, R. A. & Román, A. (1998). Activity patterns and microhabitat selection of Mallorcan midwife toad (*Alytes muletensis*) tadpoles in natural torrent pools. *Amphibia-Reptilia* **19**, 143-151.

Semlitsch, R. D. (1990). Effects of body size, sibship, and tail injury on the susceptibility of tadpoles to dragonfly predation. *Can. J. Zool.* **68**, 1027-1030.

Skelly, D. K. (1994). Activity level and the susceptibility of anuran larvae to predation. *Anim. Behav.* **47**, 465-468.

Skelly, D. K. & Werner, E. E. (1990). Behavioral and life-historical responses of larval American toads to an odonate predator. *Ecology* **71**, 2313-2322.

Waringer-Löschenkohl, A. (1988). An experimental study of microhabitat selection and microhabitat shifts in European tadpoles. *Amphibia-Reptilia* **9**, 219-236.

Warkentin, K. M. (1999). The development of behavioral defenses: a mechanistic analysis of vulnerability in red-eyed tree frog hatchlings. *Behav. Ecol.* **10**, 251-262.

Wassersug, R. (1973). Aspects of social behavior in anuran larvae. pp. 273-297. In: *Evolutionary Biology of the Anurans: Contemporary Reserach on Major Problems*. J. L. Vial (ed.). Univ. Missouri Press, Columbia.

Watt, P. J., Nottingham, S. F. & Young, S. (1997). Toad tadpole aggregation behaviour: evidence for a predator avoidance function. *Anim. Behav.* **54**, 865-872.

Werner, E. E. (1992). Competitive interactions between wood frog and northern leopard frog larvae: the influence of size and activity. *Copeia* **1992**, 26-35.

Werner, E. E. (1994). Ontogenetic scaling of competitive relations: Size-dependent effects and responses in two anuran larvae. *Ecology* **75**, 197-213.

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